

We claim:

1. A method for the production of recombinant proteins with high-mannose carbohydrate structure, comprising continuously culturing cells of *Pichia pastoris*, which cells comprise a DNA molecule which encodes a protein of interest, under conditions suitable for the expression of said DNA molecule.
2. The method of claim 1, wherein the recombinant proteins are human lysosomal enzymes selected from the group consisting of lysosomal acid lipase, alpha glucosidase, alpha-L idronidase, alpha galactosidase, iduronate sulfatase, galactosamine-6-sulfatase, beta galactosidase, and arylsulfatase B.
3. The method of claim 1, wherein the DNA molecule comprises a promoter operatively linked to a DNA coding sequence.
4. The method of claim 3, wherein the constitutive promoter is the GAPDH promoter.
5. The method of claim 4, wherein the cells are cultured without the addition of molecular oxygen.
6. A method for the production of recombinant glucocerebrosidase with high-mannose carbohydrate structure, comprising culturing cells of *Pichia pastoris* which cells comprise a DNA molecule which encodes glucocerebrosidase, under conditions suitable for the expression of said DNA molecule.
7. The method of claim 6, wherein the DNA molecule comprises a constitutive promoter operatively linked to a coding sequence for glucocerebrosidase.
8. The method of claim 6, wherein the cells are continuously cultured without the addition of molecular oxygen.
9. A method for purification of recombinant human glucocerebrosidase with high-mannose carbohydrate structure, comprising culturing cells of *Pichia pastoris* which cells comprise a

DNA molecule which encodes glucocerebrosidase, under conditions suitable for the expression of said DNA molecule to produce recombinant human glucocerebrosidase in a cell culture, and purifying said produce recombinant human glucocerebrosidase from said cell culture.

- 5 10. The method of claim 9, wherein the DNA molecule comprises a constitutive promoter operatively linked to a coding sequence for glucocerebrosidase.
11. The method of claim 9, wherein the cells are continuously cultured without the addition of molecular oxygen.
12. A method for the production of recombinant sphingomyelinase with high-mannose
10 carbohydrate structure, comprising culturing cells of *Pichia pastoris* which cells comprise a DNA molecule which encodes sphingomyelinase, under conditions suitable for the expression of said DNA molecule.
13. The method of claim 12, wherein the DNA molecule comprises a constitutive promoter operatively linked to a coding sequence for sphingomyelinase.
- 15 14. The method of claim 12, wherein the cells are continuously cultured without the addition of molecular oxygen.
15. A method for purification of recombinant human sphingomyelinase with high-mannose carbohydrate structure, comprising culturing cells of *Pichia pastoris* which cells comprise a
20 DNA molecule which encodes sphingomyelinase, under conditions suitable for the expression of said DNA molecule to produce recombinant human sphingomyelinase in a cell culture, and purifying said produce recombinant human sphingomyelinase from the cell culture.
16. The method of claim 15, wherein the DNA molecule comprises a constitutive promoter operatively linked to a coding sequence for sphingomyelinase.

17. The method of claim 15, wherein the cells are continuously cultured without the addition of molecular oxygen.

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- ✕ BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**